HERBIVORY HABITS OF BEEF COWS GRAZING NATIVE RANGE INFESTED BY SERICEA LESPEDEZA

by

GARRETT WILLIAM PREEDY

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Major Professor
KC Olson

Abstract

Our objective was to determine the effects of supplemental corn steep liquor (**CSL**) on voluntary selection of sericea lespedeza (**SL**) by beef cows grazing native tallgrass range. Dietary botanical composition of cows (n = 145; initial BW = 579 ± 91 kg) was evaluated during a 150-d grazing period (5/1 to 10/1). Native pastures (n = 9; 50 ± 17 ha) infested by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cows or grazing by cows supplemented with CSL (1.79 kg DM • cow^{-1} • d^{-1}; 45% DM, 34% CP). Cows were assigned randomly to treatment and pasture (stocking rate = 0.5 ha/AUM). Concentration and protein-binding capacity of condensed tannins (**CT**) in SL were measured monthly. Fecal samples were collected from each cow on 6/1, 7/1, 8/1, 9/1, and 10/1. Herbivory of SL was estimated along line transects in October. Plant fragments in fecal samples were quantified via a microhistological technique; fragment prevalence in fecal material was assumed to equal botanical composition of the diet. Concentration and protein-binding capacity of CT in SL were greatest ($P < 0.01$) on 8/1 and 9/1, respectively. The proportion of individual SL plants showing evidence of herbivory tended to be greater ($P = 0.09$) on pastures grazed by supplemented cows compared to pastures grazed by unsupplemented cows (94 vs. 80% of SL stems, respectively). Prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by month. Prevalence of SL in beef cow diets was not different ($P \geq 0.35$) between treatments when concentration and protein-binding capacity of CT were relatively low (6/1, 7/1, and 10/1). In contrast, supplemented cows selected more ($P < 0.01$) SL than unsupplemented cows when concentration and protein-binding capacity of CT were greatest (8/1 and 9/1). We interpreted these data to suggest that voluntary selection of SL by beef cows was inversely related to concentration of CT; moreover, supplemental CSL stimulated voluntary selection of SL during periods of high CT concentration. Supplemental CSL did not influence selection of other plant species that were monitored.

Keywords: condensed tannins, diet selection, *Lespedeza cuneata*

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Chapter 1 - Review of Literature

Introduction

Sericea lespedeza (*Lespedeza cuneata*, **SL**) has become a serious threat to the Flint Hills of Kansas and the Osage Hills of Oklahoma due to its ability to thrive in rocky terrain and reproduce prolifically (Eddy et al., 2003). The use of herbicides to kill SL or retard the rate of SL infestation has been researched heavily; however, treatment is costly and application is difficult due to terrain (Eddy et al., 2003). High levels of condensed tannins in SL deter ruminants from putting significant grazing pressure on this noxious weed (Terrill et al., 1989; Mantz et al., 2009; Eckerle et al., 2011a).

Condensed tannins are oligomeric, polyphenolic compounds that have a high affinity for dietary proteins that, once bound to tannins, form an insoluble tannin-protein complex (Villalba and Provenza, 2002). Concentration of condensed tannins in SL increases throughout the growing season with the peak concentration corresponding to the budding stage of SL growth (Eckerle et al., 2010). Deterrence of grazing during this period ensures maximum seed production (670 kg of seed/ha; Wang et al., 2008). Co-grazing with cattle and goats and goat grazing alone have increased grazing pressure on SL, thereby greatly reducing SL biomass and seed production (Mayo, 2000; Hart, 2001; Pacheco et al., 2012). Lack of facilities and additional fencing required to care adequately for goats in conjunction with the Beef Cattle Culture of the Flint Hills has prevented wide-scale adoption of this grazing system.

Several studies have been performed to evaluate the use of beef cattle as a biological control for SL (Mantz et al., 2009; Eckerle et al., 2011a, 2011b and 2001c). Supplementation with either polyethylene glycol or corn steep liquor have been effective in increasing DMI of fresh SL or SL-contaminated prairie hay in confinement; however, it has yet to be determined if dietary supplements promote voluntary selection of SL by cattle grazing SL-infested native tallgrass pastures.

Literature Review

Sericea Lespedeza

Sericea lespedeza is a protein-rich perennial legume that was introduced to Kansas in the 1950s through the Soil Bank program (Ohlenbush et al., 2001); however, wide-scale invasion by SL began after the initiation of the Conservation Reserve Program in the 1980s (Eckerle, 2011). It is estimated that > 2,500 km^2 in Kansas are infested with SL (Eddy et al., 2003). This dramatic increase is due to multiple traits that allow the plant to spread very quickly. Drought resistance, prolific seed production, high condensed-tannin content, canopy dominance, and allelopathic properties all contribute to the invasive nature of SL (Kalburtji and Mosjidis 1992; Dudley and Fick, 2003; Eddy et al. 2003; Vermeire et al., 2007).

Drought Resistance

Sericea Lespedeza was imported from Eastern Asia in 1896 (Pieters, 1939) for the purpose of soil conservation in the southeastern United States (Pieters et al., 1950). It was able to thrive in soils with poor fertility characteristics (Mosjidis, 1997) and it exhibited strong resistance to drought conditions due to its deep taproot system (Ball and Mosjidis, 2007). Sericea lespedeza was first thought to be unsuitable for areas receiving less than 89 cm of precipitation annually (Pieters, 1939; Bailey, 1951); however, SL has adapted to the Flint Hills of Kansas that average 76 to 100 cm of rainfall annually (Goodin et al., 1995). This drought resistance stems from a taproot system that can extend over 120 cm in the clay soils of Eastern Kansas (Bailey, 1951; Guernsey, 1970; Koger et al., 1996).

Seed Production

The spread of SL is also strongly related to its prolific seed-producing capabilities. Wang et al. (2008) estimated that pure SL stands could produce 670 kg of seed/ha on an annual basis, whereas Fechter (2003) estimated that SL produced up to 1000 seeds/stem. With up to 30 stems/plant (Pieters, 1939), the result could be a massive build-up of SL seed in the soil bank. Few interventions have been shown to effectively reduce SL seed production in a pasture setting; however, the use of herbicides, climatic drought, and increased grazing pressure has resulted in decreases in the production of seed. A 7 to 9% decrease in single-season stem density was reported after herbicides were applied (Koger et al., 2002); however, reduction of all broad-leaf plants (i.e., forbs) is a consequence of herbicide usage. Many of these non-target forbs are vital to stability of native range ecosystems and important components of herbivore diets (Eddy et al., 2003). Ward et al. (1985) reported that the seed production of SL was reduced by drought stress but drought also increased the condensed-tannin content (Donnelly, 1959) of SL leaves, which may intensify deterrence of grazing by cattle and sheep.

Goats reportedly have greater tolerance for condensed tannins in SL than sheep or cattle (Robbins et al., 1991). Therefore, research on multispecies grazing has increased over the last decade. Pacheco et al. (2012) reported an increase in herbivory of SL stems in pastures grazed by beef cows and goats compared to pastures grazed only by beef cows. A 4-yr goat grazing trial was conducted by Mayo (2000). Goats exhibited strong affinity for SL and, therefore, reduced SL cover by 25% during the 1st season of the study. By season 4, only trace amounts of SL remained in pastures. Seed production was also effected by grazing goats. Initially, SL produced an average of 950 seeds/stem across all pastures; however, by the 4th year, seed production had been reduced to an average of 3 seeds/stem. Mayo (2000) noted that 7.5 goats/ha were necessary to keep SL in check under the conditions of their study. Widespread use of small-ruminant grazing has not occurred in the area due to increased husbandry requirements, fencing requirements, and predator control requirements; moreover, there are significant cultural and animal-marketing challenges related to small ruminant production in the Kansas Flint Hills.

Condensed Tannins

Sericea lespedeza is relatively unpalatable late in the grazing season to sheep (Wolf and Dove, 1987) and cattle (Hoveland et al., 1969; Eckerle et al., 2011a) due to its elevated condensed tannin content and its coarse, erect stems (Hoveland et al., 1969; McGraw and Hoveland, 1995). Voluntary grazing of SL by cattle and sheep has been observed during the early portion of the grazing season when the plant is immature and has modest tannin content; however, grazing typically ceases as the plant buds (Altom et al., 1992; Wang et al., 2008).

The effects of SL intake on DMI, total-tract digestibility (DM, CP, NDF), and total-digestible DMI by mature cows were evaluated by Eckerle et al. (2011a). Tallgrass prairie hay either uncontaminated or contaminated with SL (19.3% of total biomass) were fed *ad libitum* to cows. A sharp decline in DMI was observed among cows fed contaminated hay immediately after initiation of the treatment. Within 9 d of feeding initiation, DMI of cows offered contaminated hay had decreased from 2.2% of BW to 0.4% of BW. Upon analysis of total-tract nutrient digestibilities (DM, CP, NDF), no treatment differences were observed; however, total-digestible DMI of cows consuming uncontaminated hay was greater than that of cows consuming SL-contaminated hay.

Properties of Condensed Tannins

Tannins are polyphenolic compounds that accumulate in many plants as products of secondary plant metabolism and may be divided into two groups based on chemical origin: hydrolysable tannins and condensed tannins (Caygill and Mueller-Harvey, 1999). For the purpose of this literature review, only condensed tannins will be evaluated due to their association with invasion dynamics of SL. Condensed tannins are polymers of flavan-3-ols that form pink anthocyanidins upon heating; therefore, they are sometimes referred to as proanthocyanidins. This pink color is mentioned in the condensed-tannin concentration measurement protocol described by Makkar (2003).

Tannin concentration in SL is highly variable, depending upon: location in the plant, time of the growing season, whether the plant is fresh or sun-cured, climatic conditions, and plant age. Cope and Burns (1974) reported that SL leaves were higher in condensed tannins than stems. It was speculated that condensed tannins are a defense mechanism developed by SL through natural selection to prevent herbivory (Swain, 1979). Eckerle et al. (2010) reported that condensed-tannin concentrations in SL were least during the spring and fall and greatest during summer. These researchers also indicated that sun-dried SL had lower condensed-tannin concentrations than fresh SL.

Drought and plant age affect condensed-tannin concentrations, as well. As temperature increased and precipitation decreased, condensed tannins increased in growing SL plants; likewise, mature SL plants had greater condensed-tannin concentrations than seedling plants (Donnelly, 1959). This may explain why goats prefer seedlings to mature plants in native pastures (Hart, 2001). It has been noted that even goats were noticeably deterred by SL condensed tannins when at their greatest concentration, near the middle of the growing season (Provenza et al., 1990).

Protein-Binding Capacity

Protein-binding capacity of condensed tannins is measured as the proportion of condensed tannins in a given sample that precipitate proteins (Makkar, 2003). This measure is valuable to ascertain the strength of the avoidance response exhibited by domestic herbivores maintained on SL-infested pastures. The process involved in the binding of tannins and proteins is complex and depends on quantity, structure, and size of both molecules. These complexes are semi-irreversible and are very stable in anaerobic environments such as the rumen (i.e., pH 3.5-7.0; Hagerman et al., 1992). Conversely, the tannin-protein complexes are susceptible to dissociation under acidic conditions, as occur in the abomasum, (Jones & Mangan, 1977). This condition decreases breakdown of proteins into peptides, amino acids, ammonia, and VFA in the rumen and increases passage of intact proteins to the small intestine (Reed, 1995). As ruminal escape protein increases, there is a general decrease in ruminal methane production and reduced incidence of bloat among cattle consuming forages with elevated condensed-tannin concentrations (Reed, 1995; Min et al., 2005). Unfortunately, Eckerle et al. (2011b) reported that N availability in the gut decreased to the point that total-tract CP digestibility approached zero among beef cows fed prairie hay contaminated by SL.

Positive Effects of Condensed Tannins

Multiple beneficial effects are related to the inclusion of condensed tannins in the diet including bloat prevention, reduced parasite load, and increased wool and milk yield in ewes. Bloat control was improved by adding small amounts of condensed tannins into beef cattle diets (Min et al., 2005; Rochfort et al., 2008). The tannin-protein complex prevented frothy bloat by binding bloat-provocative proteins in the rumen. Additionally, low levels of condensed tannins added to the diets of ewes increased wool production and improved reproductive performance (Min and Hart, 2003; Min et al., 2005).

Dry Matter Intake

High levels of condensed tannins ($\geq$ 5% of whole-plant DM) in SL cause a significant decrease in voluntary DMI by sheep and cattle (Eckerle et al., 2011a), while relatively modest levels of tannins in SL do not seem to affect DMI (Barry & Duncan, 1984). Dry matter intake and extent of DM digestion were decreased in sheep consuming high-tannin diets; however, no differences were observed in extent of fiber digestion, due possibly to increased ruminal retention time (Waghorn et al., 1994). Eckerle et al. (2011a) reported a sharp decrease in DMI by mature beef cows consuming high-tannin, SL-contaminated prairie hay; moreover, cows nearly stopped consuming contaminated forage within 9 d of the onset of that experiment. Polyethylene glycol + N, P, and S supplementation increased DMI by as much as 50% in sheep consuming *Acacia aneura*, a forage with elevated levels of condensed tannins (Pritchard et al., 1992).

Polyethylene Glycol

Condensed tannins have a high affinity for proteins in the diets of ruminants, resulting in formation of strong tannin-protein complexes (Villalba and Provenza, 2002). Tannins that associate with polyethylene glycol (**PEG**) before they have the opportunity to interact with dietary proteins may lose that affinity (Waghorn et al., 1987; Jones and Mangan, 1997). Condensed tannins bind preferentially to PEG, thereby preventing the formation of complexes between condensed tannins and dietary proteins (Mantz et al., 2009).This bonding is pH dependent and is greatest at a neutral pH, similar to the conditions that prevail in the rumen (Makkar, 2003). Supplementing ruminant diets with PEG has increased DMI of forages with elevated condensed-tannin concentration (Landau et al., 2002; Mantz et al., 2009). Unfortunately, feeding PEG to ruminants as a feedstuff has not been attempted commercially in the United States due to prohibitive costs and regulatory prohibition (Eckerle et al., 2011b). The Association of Feed Control Officials permits the use of PEG only as a feed additive and not as a primary feed ingredient or a supplement (AAFCO, 2008).

Corn Steep Liquor

The wet milling of corn yields several byproducts, one of which is corn steep liquor (**CSL**; Talpada et al. 1987), which is produced in excess of 590,000 metric tons annually in the United States (Hull et al., 1996; CRA, 2006). Product variability has been greatly reduced in recent years; however, some variation in chemical composition is expected because the production of byproducts from wet corn milling is not a uniform process (CRA, 2006). Corn steep liquor is regarded as a good source of protein, free amino acids, energy, vitamins, and minerals for beef cattle (Wagner et al., 1983) with a DM content of approximately 50% (Talapada et al., 1987; CRA, 2006). Kalscheur et al. (2008) reported that CSL was 44.2% CP and 2.3% NDF on a DM basis. It is relatively inexpensive, palatable, and *Generally Regarded as Safe* (GRAS) by the U.S. Food and Drug Administration (FDA, 2013).

Wagner et al. (1983) evaluated the effects of supplemental CSL on cows grazing dormant native tallgrass pastures. Greater weight gains were observed among CSL-supplemented cows compared with cows fed other supplements. Corn steep liquor increased ruminal ammonia concentrations and reduced ruminal pH levels when compared with unsupplemented cows. Cows supplemented with CSL had reduced ruminal acetate concentrations and increased ruminal butyrate and isovalerate concentrations when compared to unsupplemented cows or cows supplemented with condensed molasses solubles or fermented condensed whey. Overall, CSL appeared to be an effective protein source for beef cows grazing dormant native range.

Sericea Lespedeza Research

The use of CSL to alleviate the negative effects of SL consumption by beef cattle was evaluated by Eckerle et al. (2011b). They developed a method to evaluate the suitability of various feedstuffs as potential mitigators of dietary condensed tannins. Their method involved mixing tannic acid with bovine serum albumin (BSA) in the presence or absence of potential tannin-mitigating compounds. After the reaction was allowed to take place in the presence or absence of a mitigating agent, BSA in the solution that remained unbound by tannin was measured.

Among the mitigating agents that were evaluated were PEG and CSL. The chosen dose of the mitigating agents created a ratio of mitigator to true protein that was approximately equal to the feeding rate of PEG recommended by Mantz et al. (2009) to increase consumption of SL by beef cattle and the CP level in the diets fed by Mantz et al. (2009). In untreated samples, an average of 57.3% of BSA was bound by tannic acid and would have been resistant to ruminal microbial protein digestion. Tannin-bound protein in the PEG-treated samples declined 16% compared with untreated samples. Conversely, an equivalent dose of CSL appeared to fully protect BSA from binding by tannins.

Subsequently, a feeding trial was performed in which beef cows were fed SL-contaminated prairie hay; CSL was supplemented to these cows at 0, 0.6, 1.2, or 1.8 kg DM • cow^{-1} • d^{-1} (Eckerle et al., 2011b). Supplemental CSL fed at a rate of 0.6 kg DM/d alleviated the negative effects of condensed tannins on DMI, whereas total-tract digestion of CP and digestible DMI were normalized when CSL was fed at 1.2 or 1.8 kg DM/d. Although the negative effects of SL tannins were reduced using CSL (Eckerle et al., 2011b), it was unknown if cows would voluntarily select SL-contaminated forage if uncontaminated forage was available simultaneously. Eckerle et al. (2011c) assessed the effects of 0.6 kg DM/d of supplemental CSL on the voluntary selection of SL-contaminated and SL-free prairie hays that were simultaneously available. Corn steep liquor supplementation did not change DMI of uncontaminated hay; however, cows supplemented with CSL voluntarily ate more SL-contaminated hay and more total forage (i.e., total of SL-contaminated and SL-free hay consumption) than unsupplemented cows. Total-digestible DMI was greater also for supplemented cows compared to unsupplemented cows.

Conclusions

Over 2,500 km^2 of grasslands in Kansas are infested by SL (USDA, 2010). Herbicide treatment of SL is expensive; moreover, grassland acreage affected by SL increased over 60-fold between 1988 and 2000, in spite of routine herbicide usage during that period (Eddy et al., 2003).

Herbivory by goats reduced SL seed production significantly (Mayo, 2000; Hart, 2001); however, widespread use of goat grazing faces significant cultural and economic challenges in the Kansas Flint Hills (Pacheco et al., 2012). Elevated condensed-tannin content of SL strongly deters voluntary consumption by beef cattle (Eckerle et al., 2011a and 2011b). Increased grazing pressure on SL by beef cattle may slow its spread and facilitate a measure of biological control.

Feedstuffs with tannin-binding properties may promote voluntary consumption of SL by beef cattle. Eckerle et al. (2011b) reported that moderate amounts of supplemental corn steep liquor (CSL; 0.6 to 1.8 kg/d) normalized DMI and total-tract protein digestion by beef cows fed SL-contaminated prairie hay in confinement. In addition, beef cows supplemented with CSL at 0.6 kg DM/d did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial, whereas unsupplemented beef cows displayed strong preference for SL-free prairie hay over SL-contaminated prairie hay (Eckerle et al., 2011c).

It is unknown if supplemental CSL may influence dietary selection preferences of cattle freely grazing native tallgrass range infested by SL. Therefore, our objective was to evaluate the effects of supplemental CSL on herbivory patterns of beef cows grazing native tallgrass rangeland infested by SL in the Kansas Flint Hills.

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Chapter 2 - High-Tannin Forage Utilization by Beef Cows IV. Effects of Corn Steep Liquor Supplementation on Performance and Herbivory Patterns of Beef Cows Grazing Native Range Infested by Sericea Lespedeza (*Lespedeza cuneata)*

Abstract

Sericea lespedeza (**SL**) is classified as a noxious weed in Kansas. We previously reported that supplementation with corn steep liquor (**CSL**) increased acceptance of and tolerance for SL-contaminated hay by beef cows fed in confinement. Therefore, the objective of this trial was to determine if supplemental CSL would promote herbivory of actively-growing SL by beef cattle grazing native rangeland in the Kansas Flint Hills. Herbivory of SL and performance of lactating crossbred cows and calves (n = 145; initial BW = 579 ± 91 kg) were evaluated during a 5-mo experiment. Native tallgrass pastures (n = 9; 50 ± 17 ha) infested heavily by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cows or grazing by cows supplemented with CSL (1.79 kg DM • cow^{-1} • d^{-1}; 45% DM, 34% CP). Cow-calf pairs were assigned randomly to treatment and pasture (stocking rate = 0.5 ha/AUM). Animal BW were measured monthly from June 1 to October 1; cows were assessed for BCS at those times also. Total forage biomass and SL biomass were measured monthly. Herbivory of SL was estimated along line transects in October. Cow BW change, BCS change, and pregnancy rate were not different ($P \geq 0.22$) and calf ADG was not different ($P \geq 0.29$) between treatments. Additionally, total forage biomass and

SL biomass were not different ($P \geq 0.52$) between treatments; however, both varied ($P < 0.01$) by month of the grazing season. The proportion of individual SL plants showing evidence of herbivory tended to be greater ($P = 0.09$) on pastures grazed by supplemented cows than on pastures grazed by unsupplemented cows (94 vs. 80% of SL stems, respectively). Under the conditions of our study, beef cows supplemented with CSL appeared to put greater grazing pressure on SL compared to unsupplemented beef cows; however, animal performance was not influenced by CSL supplementation.

Introduction

Sericea lespedeza (*Lespedeza cuneata*, **SL**) is classified as an invasive plant throughout the Great Plains. It infests approximately 2,530 km^2 of grassland in Kansas (Wang et al., 2008; USDA, 2010). The aggressive nature of the plant reduces native grass production by up to 92%, through a combination of prolific seed production, canopy dominance, and allelopathy (Kalburtji and Mosjidis 1992; Dudley and Fick, 2003; Eddy et al. 2003; Vermeire et al., 2007). Herbicides retard the spread of SL but application is expensive; moreover, herbicides are lethal to ecologically-important, non-target plant species (Eddy et al., 2003). Goat herbivory reduced SL seed production significantly (Mayo, 2000; Hart, 2001); however, widespread use of goat grazing faces significant cultural and economic challenges in the Kansas Flint Hills (Pacheco et al., 2012).

Increased grazing pressure on SL by beef cattle, the most economically-relevant herbivore in the region, may slow its spread and facilitate some measure of biological control. Unfortunately, mature plants contain high levels of condensed tannins which reduce protein digestion by beef cattle and strongly deter grazing (Mantz et al., 2009).

Supplemental corn steep liquor (**CSL**) alleviated the negative effects of condensed-tannin ingestion by beef cattle fed prairie hay contaminated with SL (Eckerle et al., 2011b). In addition, beef cows supplemented with CSL did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial (Eckerle et al., 2011c). Therefore, the objective of our study was to evaluate the effects of supplemental CSL on herbivory patterns and performance of beef cows grazing native tallgrass rangeland infested by SL.

Materials and Methods

Animal care practices used in our study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978).

Location

Our study was conducted between May 1 and October 1, 2011 in Chautauqua County, KS on 9 native tallgrass pastures located approximately 16 km southeast of Sedan, KS. Pastures were burned April 10. Plant-species composition of pastures was estimated immediately before initiation of the trial using a modified step-point technique (Table 2.1; Owensby, 1973).

Weather

Climatic data were collected from a weather center near Sedan, KS (ID: USC00147305). Monthly average minimum and maximum temperatures and total monthly precipitation were contrasted against the 30-y average for this location (Table 2.2).

Animals

Lactating, crossbred beef cows with calves (n = 145; initial cow BW = 579 ± 91 kg; initial calf BW = 139 ± 32 kg) were blocked by age and calving date and assigned randomly to 1 of 2 treatments: no supplementation or supplementation with CSL. Cow and calf BW were measured at monthly intervals from June 1 to October 1. Cow BCS (scale = 1 to 9, 1 = emaciated, 9 = obese; Wagner et al., 1988) was assessed also at those times by 2 trained observers; BCS scores assigned to each cow by each observer were averaged. Cow-calf pairs were allowed to graze freely from May 1 to October 1. Cows were exposed to natural-service breeding from May 1 to July 15. Calves were weaned September 1 at an approximate age of 200 d. Cow pregnancy rates were determined via rectal palpation 75 d after bull exposure was terminated.

Treatments

Native tallgrass pastures (n = 9; 50 ± 17 ha) infested heavily by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cow-calf pairs or grazing by cow-calf pairs supplemented with CSL. Animals were assigned randomly to pastures within designated treatment groups. All pastures were stocked at 0.5 ha/AUM, a rate typical of the Kansas Flint Hills. Beginning June 1, cow-calf pairs were fed supplemental CSL that was delivered 3×/wk in portable feed bunks (61 cm bunk space/cow). Delivery of CSL was prorated for an average daily intake of 3.0 L/cow daily (i.e., 1.79 kg DM • cow^{-1} • d^{-1}). Prior research reported that 1.8 kg CSL/cow daily (DM basis) provided complete relief from the symptoms of condensed tannin consumption among beef cows fed SL-contaminated prairie hay (Eckerle et al., 2011b).

Corn steep liquor, a viscous, liquid byproduct of wet-corn milling, was purchased from Archer Daniels Midland in Columbus, NE; each truckload was sampled randomly to determine chemical composition (Table 2.3).

Herbivory

Two permanent 100-m transects were established in each pasture at the onset of the trial (June 1) in order to estimate above-ground forage biomass, botanical composition, and SL herbivory. Total forage biomass and SL biomass were estimated by clipping all live plant material from within randomly-placed sampling frames (0.25 m^2; n = 10/pasture) at a height of 1 cm on 6/1, 7/1, 8/1, 9/1, and 10/1. Forage samples were hand sorted to separate SL from all other forage plants; samples were then placed in separate paper bags to be sun-dried at the collection site for 8h. Because of the remote location of our research site, sun drying was used to prevent aerobic degradation and nutrient loss in forage samples during transport to analytical facilities. Herbivory of individual SL plants was estimated visually at the end of the study (October 1) at 5-m intervals along each transect. The closest SL plant to each point was examined for evidence of defoliation (e.g., stripped leaves and truncated stems). Plants were scored as either 0 (no evidence of defoliation) or 1 (defoliated).

Chemical Composition

Forage, CSL, and SL samples were dried in a forced air-oven (96 h, 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen. Dried, ground forage and SL samples were composited across pastures within sampling period and analyzed for DM (16 h, 105 °C), OM (8 h, 450 °C), and N (FP-528, LECO, St. Joseph, Michigan). These samples were also analyzed for NDF and ADF using procedures described by Van Soest et al. (1991; Table 2.3).

Statistical Analysis

Cow and calf performance were analyzed separately for each period as a mixed model with a 1-way treatment structure in a completely random design with subsampling (PROC MIXED; SAS Inst. Inc., Cary, NC). Pasture was the experimental unit and animal was the subsample unit. Class factors included animal, pasture, and treatment. The model statement included only the treatment fixed effect. The random statement had a term for pasture within treatment. Treatment means with standard error and treatment F-test p-value were reported for each period.

Cow pregnancy was analyzed as a generalized linear mixed model with a 1-way treatment structure in completely-randomized design (PROC GLIMMIX; SAS Inst. Inc., Cary, NC), using the binary distribution with logit-link function. Class factors included pasture and treatment. The model statement included only the treatment fixed effect. The random statement included only the effect for pasture within treatment. Treatment means with SE and F-test p-value were reported.

Forage and SL biomass were analyzed in two ways. First, to examine trends in biomass over time, data were analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design with a split-plot in time (PROC MIXED; SAS Inst. Inc., Cary, NC). Class factors included pasture, treatment, and period. The model statement included terms for the fixed effects of treatment, period, and treatment × period. The random statement included only the effect for pasture within treatment. Treatment × period effects were not detected; therefore, orthogonal polynomial contrasts were used to characterize the biomass trends for period main effect means up through order 4 (i.e., quartic). Second, data were analyzed separately for each period, analogous to the analysis for animal performance data, as a one-way treatment structure in a completely randomized design. The class factor was treatment and no random statement was used. Treatment means with standard error and treatment F-test p-value were reported for each period.

The proportion of grazed SL stems was analyzed as a generalized linear mixed model with a 1-way treatment structure in a completely-randomized design (PROC GLIMMIX; SAS Inst. Inc., Cary, NC), using the binomial distribution with logit-link function. Class factors included pasture and treatment. The model included a term for treatment only. The random statement contained terms for pasture within treatment and transect within pasture and treatment. Treatment means with standard error and treatment F-test p-value were reported.

For all analyses, means were considered different when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$.

Results and Discussion

Weather

Weather patterns in 2011 were characterized by below-normal precipitation and above-normal temperatures during the period of our study (Table 2.2). In general, there was an increase in mean daily high temperature of 0 to 5 °C from May through October; mean daily low temperatures were 6 to 10 °C greater than normal during the same period. Total precipitation in 2011 was approximately 21 cm less than the average observed from 1981 to 2010, whereas precipitation during the period of our study was approximately 60% of normal. The extent to which these abnormally warm, dry conditions influenced the results of our study is not fully known; however, Donnelly (1959) indicated that condensed tannins in SL increased under both above-normal temperatures and below-normal rainfall.

Nutrient Composition

Forage quality followed an anticipated pattern from June to October (Table 2.3); however, it was generally greater than what has been previously reported for annually-burned, native tallgrass prairie during the summer months (Umoh, 1977). Van Soest (1994) indicated that greater-than expected forage quality was typical of moderate drought, due to abnormally-slow rates of forage maturation. Forage CP concentrations were relatively high during May and October and were least during August, following the hottest, driest month of the year. Concentrations of NDF and ADF were generally the inverse of CP. Interestingly, CP and NDF concentrations in SL were generally more favorable than the average of available pasture forage on a month-by-month basis.

Herbivory

Initial, average, and final total forage biomass and SL biomass were not different ($P \geq 0.52$) between treatments (Table 2.4). As expected, CSL supplementation did not have an immediate, pasture-scale influence on SL biomass availability. Total forage biomass changed quartically ($P = 0.05$) over time, with forage availability peaking in July and October and reaching a nadir in August (Figure 2.1). Biomass of SL changed similarly (cubic effect - $P < 0.01$) over time. Biomass of SL was 37% of total forage biomass when averaged over all collection periods.

The proportion of SL plants with visual evidence of herbivory tended to be greater ($P = 0.09$) on pastures grazed by supplemented cows (94.2%) compared with pastures grazed by unsupplemented cows (80.2%; Table 2.4). Eckerle et al. (2010) established that whole-plant condensed tannins in fresh SL ranged from 16 to 23 % of plant DM during the growing season; moreover, SL condensed tannins were a strong deterrent to DMI (Eckerle et al., 2011a) and greatly reduced ruminal protein digestion (Eckerle et al., 2011b).

Prior research noted that supplementation with CSL appeared to mask or eliminate the post-ingestive consequences of condensed-tannin consumption. Supplemental CSL fed at a rate of 0.6 kg DM daily alleviated the negative effects of condensed tannins on DMI by beef cattle fed prairie hay contaminated with SL; total-tract CP digestion and digestible DMI were normalized when CSL was fed at 1.2 or 1.8

kg DM daily (Eckerle et al., 2011b). Beef cows supplemented with CSL at 0.6 kg DM/d did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial (Eckerle et al., 2011c).

Cow and Calf Performance

Initial cow BW, cow BCS, and calf BW were not different ($P \geq 0.76$) between treatments (Table 2.5). Supplementation with CSL had no detectable influence ($P \geq 0.22$) on cow BW change, cow BCS change, or calf ADG. Final cow pregnancy rates were also not different ($P = 0.99$) between the treatments. Reasons for lack of response to CSL supplementation on cow and calf performance measures were not immediately clear. We speculated that, under the hot, dry conditions of the study, CSL-supplemented cows may have substituted consumption of supplement for consumption of pasture forage. Alternatively, increased consumption of condensed tannins in SL may have limited performance of CSL-supplemented beef cows.

Cost Analysis

The cost of the CSL at the initiation of our trial was \$55.13/metric ton; cost per cow was estimated at \$26.40 for the 120-d period of our study (i.e., 4 kg CSL × 120 d × \$0.055/kg; as-fed basis).

A liquid feed-handling system and portable feed bunks (3 x 0.3 m) were purchased to store and feed the CSL at an installed cost of \$6,000. Assuming a 5-y period of depreciation, the annualized cash cost of this equipment was \$1,200.

For a 100-hd cow herd, cost for the storage system and bunks would have been \$12.00/cow annually. Delivery of CSL 3× weekly (i.e., 16 wk × 3 deliveries/wk @ \$20.00/delivery) was estimated at \$9.60/cow annually.

Under these conditions, supplementation with CSL cost an estimated \$48.00/cow annually. A commonly-used stocking rate across the Flint Hills of Kansas is 3.25 ha/cow during a 6-month summer grazing season; therefore, the cost of treating an SL infestation using CSL supplementation was estimated at \$14.77/ha annually (i.e., \$48.00 ÷ 3.25 ha). Treating SL with herbicides cost an estimated \$30 to 40/ha annually, at the time of this writing. It remains to be seen whether grazing SL with CSL-supplemented beef cattle will provide a degree of control comparable to that achieved with annual herbicide treatment.

Implications

Supplemental CSL fed at 1.79 kg DM/cow daily was associated with increased herbivory of SL during a summer grazing season; moreover, beef cow and calf performance were not negatively affected by condensed-tannin consumption under these circumstances. As expected, CSL supplementation did not have an immediate, pasture-scale influence on SL biomass availability; however, we speculated that repeated applications of CSL supplementation on SL-infested tallgrass pastures may impair seed-producing capabilities of SL.

Tables

Table 2.1 Botanical composition of native tallgrass pastures grazed from May to October

Item		Proportion
Grasses		**83.22**
Big bluestem	*Andropogon gerardii*	19.50
Little bluestem	*Schizachyrium scoparium*	16.94
Sedges	*Carex* spp.	14.11
Indiangrass	*Sorghastrum nutans*	7.88
Scribner's panicum	*Dichanthelium oligosanthes*	5.00
Tall dropseed	*Sporobolus asper*	4.94
Switchgrass	*Panicum virgatum*	2.44
Sand paspalum	*Paspalum setaceum*	2.17
Green bristlegrass	*Setaria geniculata*	1.89
Hairy grama	*Bouteloua hirsuta*	1.67
Purple top	*Tridens flavus*	1.33
Sideoats grama	*Bouteloua curtipendula*	0.50
Blue grama	*Bouteloua gracilis*	0.17
Other grasses	*n = 21*	4.68
Forbs		**14.29**
Lance-leaf ragweed	*Ambrosia bidentata*	2.38
Western ragweed	*Ambrosia psilostachya*	1.42
Grassleaf goldenrod	*Euthamia graminifolia*	1.28
Sericea lespedeza	*Lespedeza cuneata*	0.96
Heath aster	*Symphyotrichum ericoides*	0.59
Purple prairie clover	*Dalea purpurea*	0.03
Dotted gayfeather	*Liatris punctata*	*TR*
Other forbs	*n = 67*	7.63
Woody plants		**2.49**

Table 2.2 Growing season weather summary for Chautauqua County, KS (1981 to 2011) [a]

Item	April	May	June	July	August	September	October	Annual
2011								
Average high, °C	22.4	23.9	33.0	38.3	35.9	28.4	24.4	21.7
Average low, °C	15.1	18.1	26.6	30.9	28.7	20.5	15.7	14.7
Precipitation, cm	7.4	10.7	8.1	2.8	10.9	7.7	0.6	85.5
1981 - 2010 mean								
Average high, °C	20.8	25.1	29.6	33.1	33.3	28.4	21.9	20.7
Average low, °C	7.1	12.9	18.0	20.7	19.9	14.6	7.9	7.7
Precipitation, cm	10.0	16.8	14.8	8.7	7.7	10.4	10.4	106.3

[a] Location near Sedan, KS (ID: USC00147305)

Table 2.3 Nutrient composition of range forage, sericea lespedeza, and corn steep liquor available to beef cows and calves grazing native tallgrass pastures (DM basis)

Item	% DM[a]	% OM	% CP	% NDF	% ADF	% Ca	% P
Range forage							
June 1	92.0	92.6	9.1	53.1	37.2	0.91	0.11
July 1	91.7	93.0	9.2	47.3	36.5	1.08	0.08
August 1	91.8	93.0	7.3	53.5	39.0	0.95	0.08
September 1	91.9	93.3	9.9	46.3	36.3	1.02	0.10
October 1	92.1	93.7	11.1	45.4	38.5	1.09	0.12
SEM	0.05	0.10	0.03	0.55	0.56	0.024	0.005
Sericea lespedeza							
June 1	91.5	93.4	13.1	39.2	33.9	1.19	0.12
July 1	91.5	94.0	11.0	41.7	39.5	1.19	0.08
August 1	91.8	94.3	11.3	43.1	38.4	1.07	0.12
September 1	91.8	94.5	10.6	40.5	41.2	1.23	0.10
October 1	91.8	93.9	13.0	37.1	36.6	1.23	0.13
SEM	0.05	0.02	0.04	0.38	0.58	0.029	0.003
Corn steep liquor	45.1	88.1	34.4	3.1	2.0	0.11	1.90

[a] Samples were allowed to sun-dry at collection site for 8h before shipment back to laboratory

Table 2.4 Effects of corn steep liquor supplementation on range forage biomass, sericea lespedeza biomass, and sericea lespedeza herbivory by beef cows and calves grazing native tallgrass pastures

Item	Unsupplemented	Supplemented	SE	*P*
Initial total forage biomass, kg DM/ha	1852	2019	809.3	0.87
Average total forage biomass, kg DM/ha	2312	2445	866.5	0.88
Final total forage biomass, kg DM/ha	3309	4014	809.3	0.52
Initial sericea lespedeza biomass, kg DM/ha	231	310	567.7	0.92
Average sericea lespedeza biomass, kg DM/ha	703	1048	552.7	0.55
Final sericea lespedeza biomass, kg DM/ha	1939	2214	567.7	0.72
Sericea lespedeza stems grazed, % of total	80.2	94.2	6.65	0.09

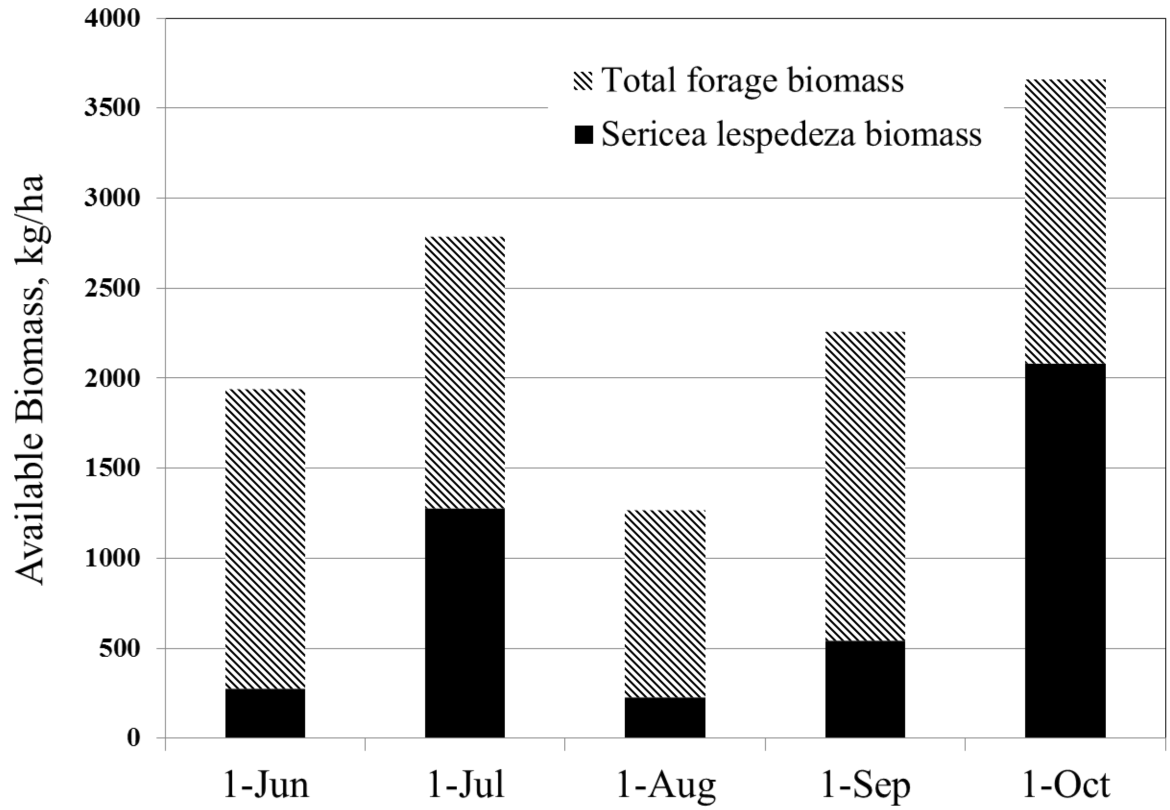
Table 2.5 Effects of corn steep liquor supplementation on performance of beef cows and calves grazing native tallgrass pastures

Item	Unsupplemented	Supplemented	SEM	*P*
Cow BW, kg				
d 0	582	577	15.2	0.84
d 60	609	614	11.3	0.77
d 121	616	629	10.4	0.36
Cow BW change, kg				
d 0-60	27.6	32.3	11.18	0.76
d 60-121	6.4	16.1	5.34	0.22
d 0-121	34.0	49.3	12.51	0.39
Cow BCS [a]				
d 0	5.3	5.2	0.18	0.76
d 60	5.4	5.4	0.11	0.84
d 121	5.0	5.1	0.05	0.27
Cow BCS change				
d 0-60	0.13	0.19	0.223	0.86
d 60-121	-0.41	-0.31	0.103	0.49
d 0-121	-0.28	-0.14	0.164	0.56
Cow pregnancy, % [b]	81.9	82.0	0.05	0.99
Calf BW, kg				
d 0	140	139	6.4	0.93
d 60	215	214	6.9	0.91
d 93	242	244	6.3	0.84
Calf ADG, kg				
d 0-60	1.23	1.24	0.052	0.91
d 60-93	0.81	0.92	0.072	0.29
d 0-93	1.09	1.12	0.033	0.52

[a] Scale = 1 to 9; 1 = emaciated, 9 = obese (Wagner et al., 1988).
[b] Pregnancy was determined via rectal palpation approximately 75 d after removal of bulls.

Figures

Figure 2.1 Total forage biomass [a] and sericea lespedeza biomass [b] on native range grazed by beef cows in the Kansas Flint Hills



[a] Total forage biomass = sericea lespedeza biomass (black portion of each bar) + biomass from all other forage plants (cross-hatched portion of each bar); quartic effect of time ($P < 0.05$).

[b] Sericea lespedeza biomass = sericea lespedeza biomass as a portion of total biomass; cubic effect of time ($P < 0.01$).

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Chapter 3 - High-Tannin Forage Utilization by Beef Cows V. Effects of Corn Steep Liquor Supplementation on Dietary Botanical Composition of Beef Cows Grazing Native Range Infested by Sericea Lespedeza (*Lespedeza cuneata)*

Abstract

Our objective was to determine the effects of supplemental corn steep liquor (**CSL**) on voluntary selection of sericea lespedeza (**SL**) by beef cows grazing native tallgrass range. Dietary botanical composition of cows (n = 145; initial BW = 579 ± 91 kg) was evaluated during a 150-d grazing period (5/1 to 10/1). Native pastures (n = 9; 50 ± 17 ha) infested by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cows or grazing by cows supplemented with CSL (1.79 kg DM • cow^{-1} •d^{-1}; 45% DM, 34% CP). Cows were assigned randomly to treatment and pasture (stocking rate = 0.5 ha/AUM). Concentration and protein-binding capacity of condensed tannins (**CT**) in SL were measured monthly. Fecal samples were collected from each cow on 6/1, 7/1, 8/1, 9/1, and 10/1. Plant fragments in fecal samples were quantified via a microhistological technique; fragment prevalence in fecal material was assumed to equal botanical composition of the diet. Concentration and protein-binding capacity of CT in SL were greatest ($P < 0.01$) on 8/1 and 9/1, respectively. Prevalence of all graminoids in beef cow diets declined ($P < 0.01$) as the grazing season advanced. Conversely, prevalence of all forbs increased ($P < 0.01$) over time. Prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by month. Prevalence of SL in beef cow diets was not different ($P \geq$

0.35) between treatments when concentration and protein-binding capacity of CT were relatively low (6/1, 7/1, and 10/1). In contrast, supplemented cows selected more (*P* < 0.01) SL than unsupplemented cows when concentration and protein-binding capacity of CT were greatest (8/1 and 9/1). We interpreted these data to suggest that voluntary selection of SL by beef cows was inversely related to concentration of CT; moreover, supplemental CSL stimulated voluntary selection of SL during periods of high CT concentration. Supplemental CSL did not influence selection of other plant species that were monitored.

Introduction

Over 2,500 km^2 of grasslands in Kansas are infested by the noxious weed, sericea lespedeza (*Lespedeza cuneata*, **SL**; USDA, 2010). Herbicide treatment of SL is expensive; moreover, grassland acreage affected by SL increased over 60-fold between 1988 and 2000, in spite of routine herbicide usage during that period (Eddy et al., 2003).

Nutrient composition of SL appears favorable for livestock production (Sidhu, 2010). Conversely, elevated condensed-tannin content strongly deters voluntary consumption of SL by beef cattle (Eckerle et al., 2011a and 2011b). Increased grazing pressure on SL by beef cattle may slow its spread and facilitate a measure of biological control.

Feedstuffs with tannin-binding properties may promote voluntary consumption of SL by beef cattle. Confined beef steers fed polyethylene glycol (PEG) ate more SL than steers not fed PEG (Mantz et al., 2009); however, use of PEG as an anti-tannin feedstuff is cost-prohibitive and disallowed from a regulatory standpoint in the US (AAFCO, 2008). Eckerle et al. (2011b) reported that moderate amounts of supplemental corn steep liquor (**CSL**; 0.6 to 1.8 kg/d) normalized DMI and total-tract protein digestion by beef cows fed SL-contaminated prairie hay in confinement. Additionally, beef cows supplemented with CSL did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial (Eckerle et al., 2011c). As an inexpensive and palatable byproduct of wet-corn milling, CSL is *Generally Regarded as Safe* by the U.S. Food and Drug Administration (FDA, 2013).

Preedy et al. (2013) indicated that SL plants tended to be more heavily defoliated in pastures grazed by CSL-supplemented cows compared to pastures grazed by unsupplemented cows; however, it was unknown if defoliation was related directly to grazing activity of cows. Therefore, our objective was to evaluate the effects of supplemental CSL on botanical composition of the diets of beef cows grazing native tallgrass rangeland infested by SL in the Kansas Flint Hills.

Materials and Methods

Animal care and handling practices used in our study were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 2978).

Location

Our study was conducted between May 1 and October 1, 2011 in Chautauqua County, KS on 9 native tallgrass pastures located approximately 16 km southeast of Sedan, KS. Pastures were burned April 10. Plant-species composition of pastures was estimated immediately before initiation of the trial using a modified step-point technique (Owensby, 1973) and presented in a previous publication (Table 1; Preedy et al., 2013). Frequently-occurring graminoids included big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), sedges (*Carex* spp.), and indiangrass (*Sorghastrum nutans*). Frequently-occurring forbs included ragweeds (*Ambrosia* spp.), grassleaf goldenrod (*Euthamia graminifolia*), and sericea lespedeza (*Lespedeza cuneata).*

Treatments

Nine pastures (50 ± 17 ha) infested heavily by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cow-calf pairs or grazing by cow-calf pairs supplemented with CSL (45% DM, 34.4% CP). All pastures were stocked at 0.5 ha/AUM, a rate typical of the Kansas Flint Hills. Cow-calf pairs were fed supplemental CSL that was delivered 3× / wk in portable feed bunks (61 cm bunk space / cow) beginning on June 1; CSL was fed to achieve an average intake of 3.0 L • cow^{-1} • d^{-1} (i.e., 1.79 kg DM/cow daily). Eckerle et al. (2011b) reported that 1.8 kg CSL • cow^{-1} • d^{-1} (DM basis) relieved symptoms of condensed-tannin consumption among beef cows fed SL-contaminated prairie hay. Chemical composition of CSL (Archer Daniels Midland, Columbus, NE) was reported previously (Preedy et al., 2013).

Animals

Lactating, crossbred beef cows with calves (n = 145; initial cow BW = 579 ± 91 kg; initial calf BW = 139 ± 32 kg) were blocked by age and calving date and assigned randomly to treatments (no supplementation or supplementation with CSL) and to pastures. Cow-calf pairs were allowed to graze assigned pastures freely from May 1 to October 1. Cows were exposed to natural-service breeding from May 1 to July 15. Calves were weaned September 1 at an approximate age of 200 d. Cow pregnancy rates were determined via rectal palpation 75 d after bull exposure was terminated. Cattle performance data were reported previously (Preedy et al., 2013). Beef cows were gathered on 6/1, 7/1, 8/1, 9/1, and 10/1, individually restrained in a squeeze chute (~ 2 min), and fecal-grab samples were collected from each animal. Each grab sample was hand-mixed to ensure homogeneity and a 40-g subsample was retained for analysis. Grab samples were sealed in plastic containers, immediately placed on ice, and transported to Kansas State University. Samples were stored frozen (-20 °C) until microhistological analysis was performed.

Microhistology

Sample preparation was conducted as described by Eckerle et al. (2009). Wet fecal samples were soaked overnight in 50% ethanol (v/v). After soaking, ethanol was decanted and samples were homogenized and washed with deionized H_2O through a No. 200 US-standard sieve. Samples were then re-homogenized, strained, and dried in a forced air-oven (96 h; 50 °C). Dried samples were ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen and stored in plastic bags for slide preparation (Bennett et al., 1999).

Slide preparation methods were described by Eckerle et al. (2009). Subsamples (0.5 g) of dried, ground, and washed plant fragments recovered from fecal material were soaked in deionized H_2O for 1 h. Approximately 20 mL of NaOH (0.05M) was then added to each sample. Samples were incubated for 20 min at room temperature to destroy plant pigments. Samples were subsequently rinsed with deionized H_2O over a No. 200 US-standard sieve and then homogenized in a blender with 20 mL of deionized H_2O for 1 min. Samples were rinsed a second time with deionized H_2O over a No. 200 US-standard sieve.

Homogenized samples were placed on slides using an eyedropper, 2 drops of Hertwig's solution were applied, and slides were placed over a propane flame until dry. Two drops of Hoyer's solution were added to each slide, a cover slip was mounted, and slides were placed over a propane flame to set the cover slip. The cover slip was then rimmed with Hoyer's solution to ensure a solid mount. Slides were dried in a forced-air oven (96 h, 50 °C) before viewing.

Individual forage-plant species previously identified as having significance in diets of beef cattle grazing in the Kansas Flint Hills (Eckerle et al., 2009; Aubel et al., 2011) were sampled before the initiation of the trial for the purpose of developing standard slides. These species included big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), sideoats grama (*Bouteloua curtipendula*), blue grama (*Bouteloua gracilis*), switchgrass (*Panicum virgatum*), indiangrass (*Sorghastrum nutans*), leadplant (*Amorpha canescensi*), heath aster (*Symphyotrichum ericoides*), dotted gayfeather (*Liatris punctata*), purple prairie clover (*Dalea purpurea*), and sericea lespedeza (*Lespedeza cuneata*). Each sample was verified as a pure forage sample before being dried in a forced-air oven (96 h, 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen.

Sample slides and standards were viewed on a compound microscope at 10× magnification. The microscope was equipped with a digital camera; 20 randomly-selected slide fields from each sample and each standard were photographed (Eckerle et al., 2009).

Photographs of standard slide fields were used to train observers to recognize histological characteristics of key plant species. Observers were required to achieve an acceptable repeatability (≤ 4% CV for each species) of plant-fragment identification relative to trainers (Holecheck and Gross, 1982).

Photographs of sample slide fields were used to measure the frequency with which plant fragments appeared in beef-cow feces (Holechek and Vavra, 1981). Individual plant species were identified according to their histological characteristics using standard slides for comparison. Due to histological similarities, big bluestem and little bluestem were grouped together for the purposes of analysis.

Plant fragment prevalence in slide fields was assumed to be equivalent to prevalence in fecal samples and equivalent to % botanical composition of the diets grazed by beef cows (Sparks and Malechek, 1968). Plant fragments that were not among the 11 range-plant species for which standards were prepared were classified as either unknown grass or unknown forb.

Extraction of Condensed Tannins

Whole-plant SL samples were collected randomly from each pasture (n = 40 plants/pasture) on 6/1, 7/1, 8/1, 9/1, and 10/1. Stems were clipped 1-cm above the soil surface, placed in paper bags, and sun-dried (8 h) at the collection site to prevent aerobic deterioration and nutrient losses. Samples were transported to an analytical facility within 24 h of collection for analysis of condensed tannins (CT) and CT protein-binding capacity. At the laboratory, SL stems were dried in a forced-air oven (96 h, 50 °C), weighed, ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1 mm screen, and composited within collection date.

Extraction of CT was conducted as described by Makkar (2003). Each ground SL sample was mixed thoroughly and a 200 mg subsample was collected. Ten mL of 50% methanol (v/v) was added to each sample in a 50 mL beaker and the mixture was stirred. Samples were agitated in an ultrasonicator (Blackstone Ultrasonics, Sheffield, PA) for 2 × 10-min periods. Samples were allowed to stand for a period of 5 min between agitations. The resulting solution was transferred to 15 mL polyethylene tubes and centrifuged at 3,000 × g (4 °C) for 15 min. The supernatant was decanted into a 50 mL beaker and chilled; the pellet was washed 2× with 5 mL of 50% methanol (v/v). The centrifugation step was repeated after each wash and supernatant was decanted into the aforementioned 50 mL beaker. All supernatant from each collection date was stored frozen (-20 °C) until further CT analysis was completed.

Measurement of Condensed Tannins

Methods used for measuring CT in harvested SL were conducted as described by Makkar (2003). A 0.5 mL aliquot of supernatant from each sample was placed into individual 5 mL conical tubes; 3.0 mL of butanol-HCl and 100 µL of ferric-chloride reagent were added and the tubes were vortexed. Samples were incubated for 60 min in a

100 °C water bath. Samples were allowed to cool and then placed in a 96-well microplate. Sample absorbance was measured at 550 nm using a UV spectrophotometer equipped with Gen5 software (Biotech Inc., Winooski, VT). Absorbance was adjusted to CT concentration using leucocyandin as a standard.

Measurement of Protein-Precipitable Phenolics

Estimation of CT protein-binding capacity in harvested SL was conducted as described by Makkar (2003). Standards (1 mL) containing 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mL tannic acid in 50% methanol (v/v) were prepared from a standard solution (0.5 mg tannic acid/mL in 1% SDS); 3 mL SDS-TEA and 1 mL ferric-chloride reagent were added to each standard (total volume = 5 mL). Absorbance was measured at 510 nm using a UV spectrophotometer equipped with Gen5 software (Biotech Inc., Winooski, VT).

Tannin-protein complexes were constructed by mixing aliquots of extracted CT (0.1, 0.2, 0.3, 0.4, and 0.5 mL) with 2 mL of bovine serum albumin solution (**BSA**; 100 mg BSA in 100 mL acetate buffer); enough 50% methanol (v/v) was added to bring the total volume to 3 mL and the solution was vortexed.

Samples were allowed to stand at 4 °C for 16 h and then centrifuged for 10 min at 3000 x g (4 °C). Supernatant was discarded and the pellet was dissolved in 1.5 mL of 1% SDS. A 1 mL aliquot was removed from each sample and mixed with 3 mL of SDS-TEA and 1-mL of ferric-chloride reagent. Iron in the form of ferric chloride reacted with tannin phenolics to express a pink chromatophore that was measurable spectrophotometrically (Makkar, 2003). The resulting solution was allowed to stand at room temperature for 30 min before being placed into a 96-well microplate.

Absorbance was measured at 510 nm. Absorbance was then converted to a tannic-acid equivalent, using the standard curve. Values were multiplied by 1.5 (each sample was dissolved in 1.5 mL of 1% SDS solution) to calculate the amount of tannin in the tannin-protein complex (Makkar, 2003). A linear regression between tannins precipitated (as a tannic-acid equivalent) and mg of SL in the original aliquot was constructed. Protein-precipitable phenolics were represented by the slope of the line.

Total phenolics in SL were measured by mixing aliquots of extracted CT (0.1, 0.2, 0.3, 0.4, and 0.5 mL) and enough 1% SDS solution to make a 1 mL final volume. This solution was added to 3 mL SDS-TEA and 1 mL ferric-chloride reagent in a 5 mL conical vial and vortexed. Absorbance was read immediately thereafter at 510 nm; absorbance was converted to a tannic-acid equivalent using the standard curve. A linear regression between tannic-acid equivalent and mg of SL in the original aliquot was constructed. Total phenolics were represented by the slope of the line.

The proportion of total phenolics which precipitated protein (i.e. protein-binding capacity) was calculated as *protein-precipitable phenolics* divided by *total phenolics*. Protein binding capacity was represented by (slope X / slope Y) * 100 (Makkar, 2003).

Statistical Analysis

Microhistology data were analyzed separately for each plant species or plant group (e.g., grasses). Data were analyzed as a generalized mixed model with a 1-way treatment structure in a completely-randomized design with subsampling and a split-plot in time (PROC GLIMMIX; SAS Inst. Inc., Cary, NC), using the binomial distribution and logit-link function. Pasture was the experimental unit and animal was the subsample unit. Class factors included animal, pasture, treatment, and collection date. The model statement included terms for the fixed effects of treatment, collection date, and treatment × collection date, which were tested using type-3 F tests. The random statement had terms for pasture within treatment, animal within pasture and treatment, and collection date × pasture within treatment.

Concentration and protein binding capacity of CT in SL were analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design with a split-plot in time (PROC MIXED; SAS Inst. Inc., Cary, NC). Pasture was the experimental unit. Class factors included pasture, treatment, and collection date. The model statement included terms for the fixed effects of treatment, collection date, and treatment × collection date, which were tested using type-3 F tests. The random statement had a term for pasture within treatment.

Treatment main effects and treatment × collection period effects were not detected ($P > 0.05$) for CT concentrations in SL, protein binding capacity of CT in SL, or for 10 of the 11 plant species examined in the microhistological portion of the study; therefore, main effects of collection date were reported. Conversely, prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by collection date; therefore, treatment × collection date means were presented. Interaction effects were tested using collection date contrasts (PROC GLIMMIX, SAS Inst. Inst., Cary, NC) according to the following equation: $(\mu_{\text{supplemented, June}} - \mu_{\text{unsupplemented, June}}) - (\mu_{\text{supplemented, date x}} - \mu_{\text{unsupplemented, date x}}) = 0$.

Least Squares collection-date main effect means were separated using the method of Least Significant Difference when protected by a significant F-test ($P < 0.05$). Means were considered different when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$.

Results and Discussion

Condensed Tannin Analysis

Concentration of CT in SL increased ($P < 0.01$) as the grazing season advanced and reached a peak during the 8/1 collection (Table 3.1). Thereafter, CT concentration declined. Protein-binding capacity of CT in SL generally followed CT concentration; however, peak protein-binding capacity occurred one month later (9/1) than peak CT concentration. Our values for CT in dried SL were greater than those reported by Eckerle et al. (2010). Weather patterns during our study were characterized by below-normal precipitation and above-normal temperatures (Preedy et al., 2013). Donnelly (1959) indicated that CT in SL increased under both above-normal temperatures and below-normal rainfall.

Microhistology

Prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by collection period (Figure 3.1). Although SL selection by CSL-supplemented beef cows was numerically greater than that by unsupplemented beef cows at each of the 5 collection dates, prevalence of SL in beef cow diets was sufficiently variable that no difference ($P \geq 0.35$) between treatments was detected when concentration and protein-binding capacity of CT were relatively low (6/1, 7/1, and 10/1; Table 3.1). Conversely, CSL-supplemented cows selected 30% more ($P < 0.01$) SL during the 8/1 collection than unsupplemented cows and 49% more ($P < 0.01$) SL during the 9/1 collection than unsupplemented cows. These times corresponded to greatest CT concentration and CT protein-binding capacity in SL.

We reported previously that supplemental CSL fed at a rate of 0.6 kg DM daily alleviated the negative effects of condensed tannins on DMI by beef cows fed prairie hay contaminated with SL; total-tract digestion of CP and digestible DMI were normalized when CSL was fed at 1.2 to 1.8 kg DM daily (Eckerle et al., 2011b). In addition, beef cows supplemented with CSL at 0.6 kg DM/d did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial, whereas unsupplemented beef cows displayed strong preference for SL-free prairie hay over SL-contaminated prairie hay (Eckerle et al., 2011c).

The relative abundance of SL in the diets of beef cows in our study ranged from a low of 3.5% (unsupplemented cows on 9/1) to a high of 7.5% (CSL-supplemented cows on 10/1). We interpreted this to indicate that, under the conditions of our study, SL was an important forb component of the diet in both supplemented and unsupplemented beef cows. The significance of increased voluntary selection of SL by CSL-supplemented beef cows during August and September was that these months corresponded to flowering and seed production by SL in the Kansas Flint Hills (Eckerle et al., 2010). Increased grazing pressure achieved with goats during this interval of time resulted in drastically-reduced seed production by SL (Mayo, 2000; Hart, 2001).

Supplemental CSL had no influence ($P \geq 0.10$) on voluntary selection of other plant species that were examined in our study; however, there were important temporal differences in selection of various grasses and forbs. Leadplant was not detected in fecal samples and was, therefore, not discussed.

Displayed preferences for individual forage plants are influenced by herbivore perceptions of palatability, plant growth form, plant nutrient composition, and herbivore experience; moreover, preferences may change dramatically over time in native-range production systems due to temporal fluctuations in availability of key species and maturity-driven changes in palatability, growth form, and nutrient content (Vallentine, 1990; Holecheck et al., 2001). In general, there were 3 distinct temporal responses in selection of the plant species we examined in our study: a decrease over time, an increase over time, or an increase from the beginning to the midpoint of the study and decline thereafter.

Voluntary selection of all grass species (Figure 3.2), big bluestem + little bluestem, indiangrass (Figure 3.3), and unidentified grasses (Figure 3.4) decreased ($P < 0.01$) from 6/1 to 10/1. We speculated that selection of these species was inversely related to nutrient content, as native tallgrasses tend to have excellent nutrient profiles while vegetative; however, quality rapidly declines as plant maturity advances (Umoh, 1977). Aubel et al. (2011) reported similar trends among beef cows grazing annually-burned native range in the Kansas Flint Hills.

During the same interval, voluntary selection of all forbs (Figure 3.2), switchgrass, blue grama, heath aster (Figure 3.5), and unidentified forbs (Figure 3.4) increased ($P < 0.05$). We speculated that selection of these plants may have increased over time due to increasing availability and high relative forage quality as the grazing season advanced. In general, the magnitude of change in forb selection from the beginning to the end of the grazing season was greater than that reported by McGinty et al. (1983) or Aubel et al. (2011). The abnormally-warm, dry conditions under which our study was conducted may have influenced both the relative availability and relative quality of the forb plants we monitored.

Voluntary selection of sideoats grama, purple prairie clover, and dotted gayfeather was relatively low at the outset of the study (Figure 3.6). Selection of each of these plant species increased as the grazing season advanced, reached a peak in August or September, and then declined thereafter. Aubel et al. (2011) reported a similar temporal pattern in selection of these 3 species but much greater prevalences in the diets of beef cows grazing native tallgrass range during a non-drought year. The drought conditions under which our study was conducted likely influenced the availability and palatability of most plant species; moreover, there were notable differences in plant composition between their research site and ours (Towne and Owensby, 1984).

Implications

Supplemental CSL increased beef cow tolerance for and acceptance of high-CT SL in a commercial-scale, native-range production system. We concluded that supplemental CSL allowed for a desirable change in selection preference by beef cows that stemmed from a critical modification of the post-ingestive consequences associated with CT consumption. The significance of increased voluntary selection of SL by CSL-supplemented beef cows during August and September was that these months corresponded to flowering and seed production by SL in the Kansas Flint Hills. Increased grazing pressure during this interval of time may result in drastically-reduced seed production by SL.

Supplemental CSL did not influence voluntary selection by beef cows of any other forage-plant species monitored in our study; however, there were noteworthy temporal shifts in selection. We speculated that these shifts in voluntary selection were driven by changes in plant availability, changes in plant nutrient composition, or both.

Tables

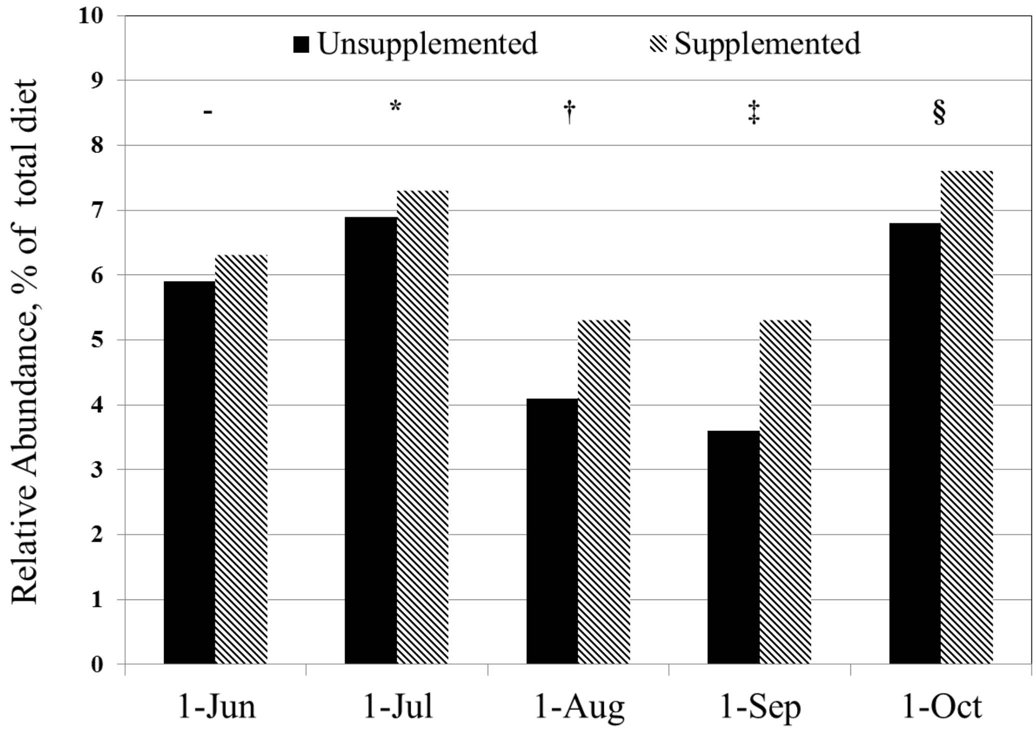
Table 3.1 Effect of harvest date on concentration and protein-binding capacity of condensed tannins in sericea lespedeza

Item	Condensed tannins, g/kg	Protein binding capacity, %
June 1	103.9[a]	46.2[a]
July 1	151.1[b]	45.5[a]
August 1	191.1[d]	49.2[c]
September 1	169.4[c]	52.3[d]
October 1	145.4[b]	47.9[b]
SEM	1.05	0.15

[a] Within a column, means without a common superscript differ ($P < 0.01$).
[b] Proportion of total phenolic compounds which precipitated proteins.

Figures

Figure 3.1 Effects of corn steep liquor supplementation on the relative abundance of sericea lespedeza[a] in diets of beef cows grazing native range in the Kansas Flint Hills



[a] SEM = 1.01

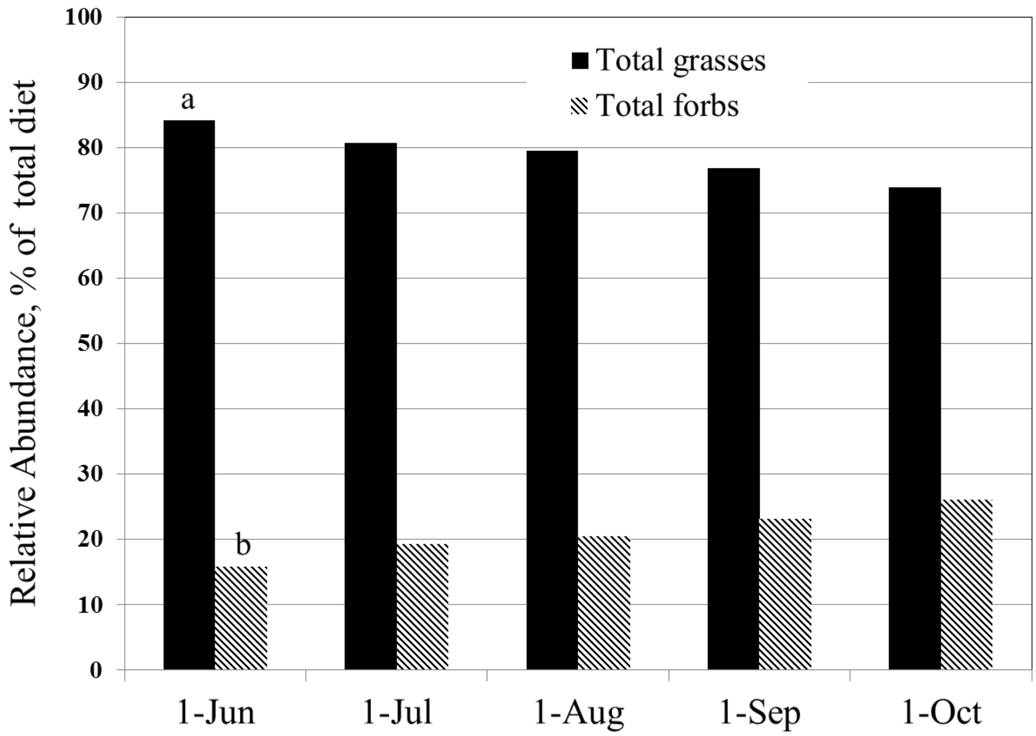
[*] Contrast: $(\mu_{\text{supplemented, June}} - \mu_{\text{unsupplemented, June}}) - (\mu_{\text{supplemented, July}} - \mu_{\text{unsupplemented, July}}) = 0$ – ($P = 0.93$).

[†] Contrast: $(\mu_{\text{supplemented, June}} - \mu_{\text{unsupplemented, June}}) - (\mu_{\text{supplemented, August}} - \mu_{\text{unsupplemented, August}}) = 0$ – ($P < 0.01$).

[‡] Contrast: $(\mu_{\text{supplemented, June}} - \mu_{\text{unsupplemented, June}}) - (\mu_{\text{supplemented, September}} - \mu_{\text{unsupplemented, September}}) = 0$ – ($P < 0.01$).

[§] Contrast: $(\mu_{\text{supplemented, June}} - \mu_{\text{unsupplemented, June}}) - (\mu_{\text{supplemented, October}} - \mu_{\text{unsupplemented, October}}) = 0$ – ($P = 0.35$).

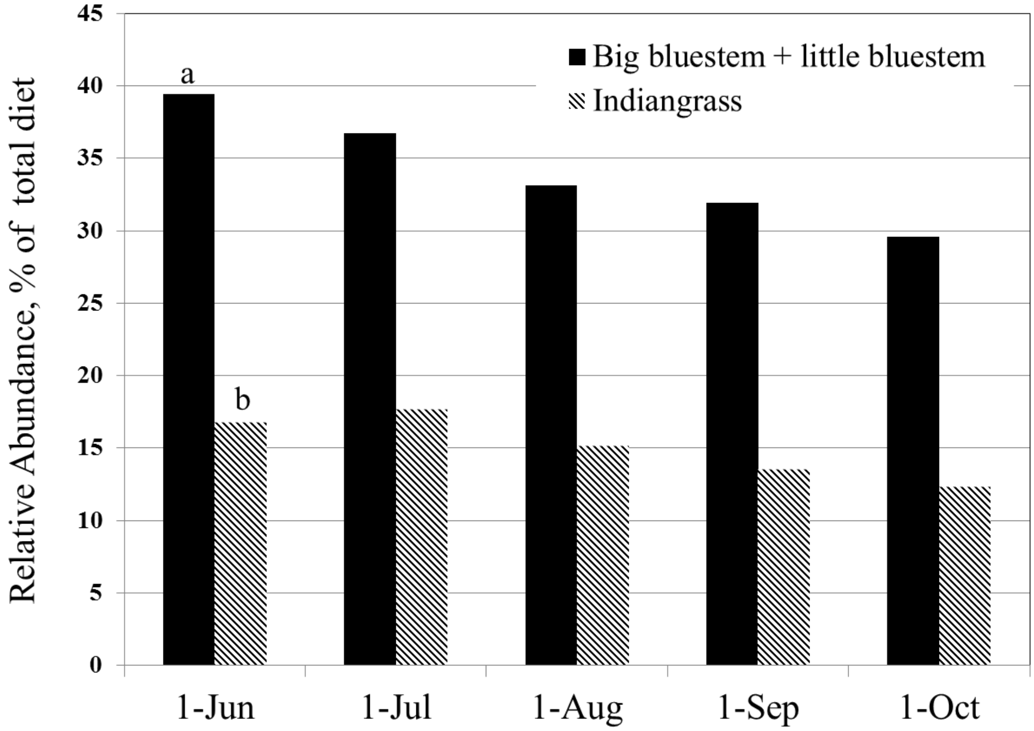
Figure 3.2 Relative abundance of grasses and forbs in diets of beef cows grazing native range in the Kansas Flint Hills



[a] Effect of time on selection of all graminoid species (Quartic – $P < 0.01$; SEM = 0.53).

[b] Effect of time on selection of all forb species (Quartic – $P < 0.01$; SEM = 0.53).

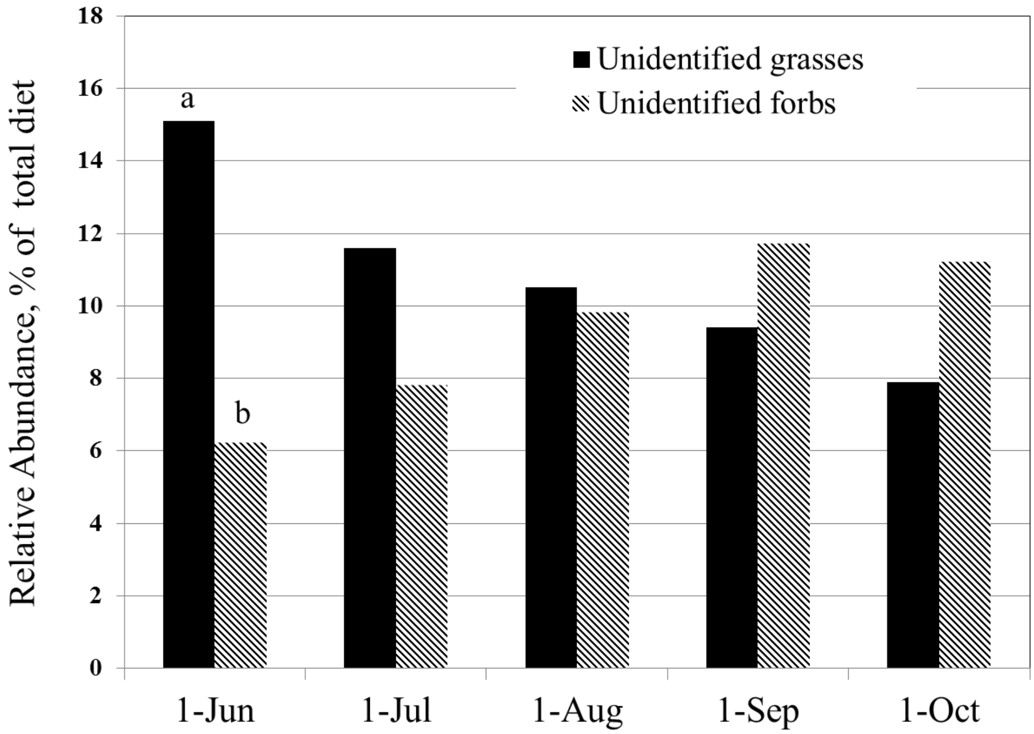
Figure 3.3 Relative abundance of bluestem *spp.* and indiangrass in diets of beef cows grazing native range in the Kansas Flint Hills



[a] Effect of time on selection of big bluestem and little bluestem (Quartic – $P < 0.01$; SEM = 0.75).

[b] Effect of time on selection of indiangrass (Quartic – $P < 0.01$; SEM = 1.05).

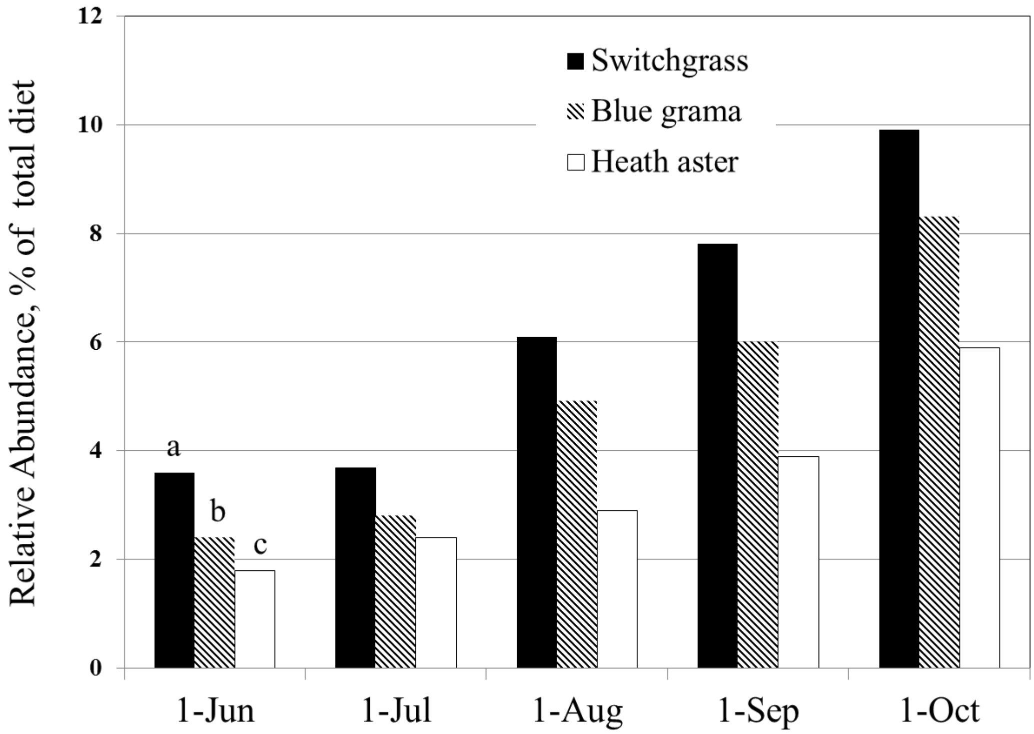
Figure 3.4 Relative abundance of unidentified grasses and unidentified forbs in diets of beef cows grazing native range in the Kansas Flint Hills



[a] Effect of time on selection of unidentified graminoid species (Cubic – $P < 0.01$; SEM = 0.45).

[b] Effect of time on selection of unidentified forb species (Cubic – $P < 0.01$; SEM = 0.34).

Figure 3.5 Relative abundance of switchgrass, blue grama, and heath aster in diets of beef cows grazing native range in the Kansas Flint Hills

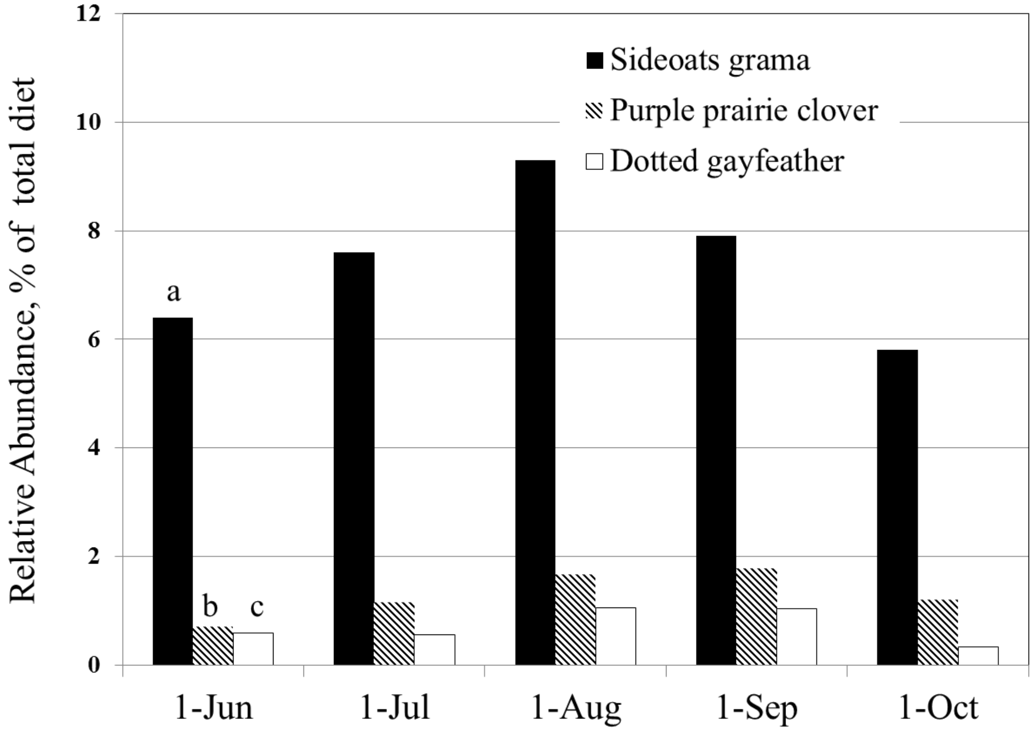


[a] Effect of time on selection of switchgrass (Quartic – *P* < 0.05; SEM = 0.96).

[b] Effect of time on selection of blue grama (Quartic – *P* < 0.01; SEM = 0.69).

[c] Effect of time on selection of heather aster (Cubic – *P* < 0.05; SEM = 0.34).

Figure 3.6 Relative abundance of sideoats grama, purple prairie clover, and dotted gayfeather in diets of beef cows grazing native range in the Kansas Flint Hills



[a] Effect of time on selection of sideoats grama (Quartic – $P < 0.01$; SEM = 0.46).

[b] Effect of time on selection of purple prairie clover (Cubic – $P < 0.05$; SEM = 0.23).

[c] Effect of time on selection of dotted gayfeather (Cubic – $P < 0.05$; SEM = 0.03).

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